

ORIGINAL RESEARCH ARTICLE



Level of selected toxic elements in meat, liver, tallow and bone marrow of young semi-domesticated reindeer (*Rangifer tarandus tarandus* L.) from Northern Norway

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Objectives. To gain knowledge on toxic elements in semi-domesticated reindeer and their distribution in meat, liver, tallow and bone marrow. The correlations between concentrations in meat and liver, as well as the use of the latter as an indicator for toxic elements in meat, were also investigated.

Study design. Cross-sectional study on population of semi-domesticated reindeer from 2 northern Norwegian counties (Finnmark and Nordland).

Methods. Semi-domesticated reindeer carcasses ($n=31$) were randomly selected, from which meat, liver, tallow and bone marrow samples were collected. Selected toxic elements (cadmium, lead, arsenic, nickel and vanadium) were studied.

Results. Liver was the organ with the highest level of all elements except for nickel, which was highest in bone marrow. Meat had the lowest levels, whereas levels in tallow and bone marrow were between those of meat and liver. Concentrations of cadmium, lead and arsenic were significantly different ($p < 0.05$) between meat and liver, while only arsenic and cadmium were significantly correlated in meat ($r_s = 0.71$, $p < 0.01$) and liver ($r_s = 0.72$, $p < 0.01$). The cadmium level exceeded the European Commission's (EC) maximum level set for bovine meat and liver in 52% of the liver samples ($n=29$). Nevertheless, the estimated monthly cadmium intake from liver of 2.29 $\mu\text{g/kg}$ body weight was well below the provisional tolerable monthly intake of 25 $\mu\text{g/kg}$ body weight set by the FAO/WHO Joint Expert Committee on Food Additives.

Conclusions. Based on the measured levels and their relation to the maximum level and to the provisional tolerable weekly/monthly intake limits, it could be inferred that consumption of reindeer meat is not associated with any health risk related to the studied toxic elements for consumers.

Keywords: *reindeer; edible tissues; cadmium; lead; arsenic; nickel; vanadium*

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Knowledge on toxic element concentrations in reindeer as terrestrial animals is necessary for assessing the potential effect in humans from the consumption of contaminants in these animals. Moreover, there has been an increasing awareness among consumers with concern to food safety and environmental toxicant issues over the past 20 years. Toxic elements (e.g. mercury, cadmium, lead and arsenic) have been an issue in Arctic terrestrial animals. The levels of persistent organic pollutants (POPs) in the

Arctic terrestrial animals are low (1). Even though contaminant levels are low in the meat of Arctic terrestrial animals, it is important to monitor their levels regularly because of high concentrations reported in their liver and kidneys. Liver has been reported to have higher concentrations of toxic elements than meat and is considered to be a good indicator of such elements in animal's body (2,3). Therefore, liver is used in food safety control routines as an indicator of toxic elements in meat.

Toxic elements are chemicals that are persistent and not metabolized, although their chemical forms may change as they pass through the intestinal tract or during storage in animal tissues (4). They are regarded as toxic to living organisms since they have tendency to accumulate in selected human and animal target tissues with the potential of causing nephrological, carcinogenic, teratogenic and immunological disorders (5,6). Toxic elements are capable of being transported over long distances (thousands of kilometres) or may be deposited near their source of origin, thereby having a local impact (1). The long range transportation of toxic elements through the atmosphere mainly depends on the size and composition of particles with which toxic elements are associated as well as their solubility.

Reindeer feed on vascular plants and lichens throughout the year with largest amount of lichens, which account for more than 50% of the winter diet for reindeer (7–9). Lichens naturally contain low amounts of essential elements (e.g. zinc and manganese) when compared with grass and are highly dependent on atmospheric supplementation in covering the reindeer's need for essential elements. Thus, high levels of such elements in lichen tissues are due to atmospheric contamination (10). The long survival, tolerability of high element concentrations and fairly uniform morphology of lichens allow for the absorption and accumulation of contaminants over the entire surface of the lichens make it suitable as a biomarker for atmospheric toxic element pollution (11,12). The availability of lichens as the reindeer's main winter feed has been described as a key factor for determining the level of heavy metals (13).

Toxicological terms such as the provisional tolerable weekly/monthly intake (PTWI/PTMI) limits set by the Joint Food and Agriculture Organization (FAO)/WHO Expert Committee on Food Additives (JECFA), as well as the maximum levels (ML) for certain contaminants set by the European Commission (EC), are relevant in relation to toxic element concentrations in both the human body and foodstuffs, respectively (14–17). The PTWI/PTMI limits are end points used for contaminants such as toxic elements with cumulative properties, representing permissible human weekly/monthly exposure to those contaminants that are unavoidably associated with the consumption of contaminated foodstuffs. The ML represents the maximum concentration of contaminants in foodstuffs in which no human health risk is associated, i.e. the concentrations that are acceptable in or on foodstuffs (17).

Our main purpose was to study the level of toxic elements in the meat, liver, tallow and bone marrow of reindeer, particularly the association between liver and meat concentrations. Additionally, we wanted to relate our results on toxic elements in meat and the rest of the studied tissues to the EC's maximum level (ML) and

FAO/WHO–JECFA's PTWI/PTMI limits available for certain toxic elements.

Materials and methods

Sample collection

Muscle, liver, tallow and bone marrow samples were randomly collected from semi-domesticated reindeer carcasses ($n = 31$ animals) from Finnmark and Nordland Counties in North Norway. The average age of the reindeer from which the samples were collected from September 2004 to January 2005 was young (1.5 years old) according to internal procedures (not published). However, a limited number of calves (approximately 6–10 months old, $n = 6$) and adult animals (over 2 years old, $n = 3$) had to be chosen because of a scarcity of slaughtered animals in the age of 1.5 years. Age of the reindeer was obtained directly from the tags attached to animals' carcasses when they passed the weighing post in the slaughterhouses.

Meat samples were collected from the muscles in the dorsal neck region. Liver samples were collected from the main loop, tallow from the fat tissue surrounding kidneys and bone marrow from the hind and front legs. All of the samples were collected immediately after the slaughter/dressing/carcass weighing process in plastic bags, though prior to being further divided into different acid-rinsed glasses according to the sample type (meat, liver, tallow or bone marrow). Samples were put on ice (approximately 4°C) immediately after collection and distribution into dedicated containers and kept frozen at –20°C (within 12 hours from the sample collection) until analysis.

Chemical analysis

The samples were separately decomposed using a microwave oven technique and concentrated supra-pure HNO_3 (5 ml) and H_2O_2 (3 ml) were added to the sample (0.6–0.7 g) before microwave oven treatment. The following temperature regimes were subsequently used in the microwave oven: 20–50°C (5 min), 50–100°C (10 min), 100–180°C (5 min) and 180°C (15 min). After cooling down the heated decomposed sample, the solution was diluted to 50 ml. The sample solution was analysed using an inductively coupled plasma high resolution mass spectrometer (ICP-HRMS), Thermo Scientific Finnigan Element-2, Germany.

All standards and calibration solutions contained 1 ppb rhenium (Re) as the internal standard, together with 1% nitric acid (HNO_3). The calibration curve was verified by a standard quality control (QC) sample, National Institute of Standards and Technology (NIST), USA. The resolutions used for elements were low [at 10] for (cadmium and lead), middle [at 20] for (vanadium and nickel) and high [at 30] for arsenic. The lens adjustment

was optimized daily to ensure maximum intensity and top separation. The analyses were done by the NILU (Norwegian Institute for Air Research) Laboratory, Kjeller, Norway. The laboratory is accredited for the methods used in the analyses according to NS-EN ISO/IEC 17025, No. TEST 008. The limits of detections (LODs) for the studied toxic elements were 3 times the standard deviation (SD) of the laboratory blanks, whereas the limits of quantifications (LOQs) were 10 times the SD of the blanks.

Statistical analysis

STATA/SE 11.0 for Windows (STATA Corp. College Station, TX, USA) was used for statistical analyses. The results were presented as a percentage of the detected toxic elements, mean, median and range. Laboratory results for metals below the LOD were given a numeric value at half the detection limit (LOD/2) according to accepted statistical practice (18). Elements concentrations were positively skewed (skewed to the right); therefore, the nonparametric Wilcoxon matched-pairs signed-rank test was used to test for significance differences in element concentrations between meat–liver and tallow–bone marrow. A Spearman's rank correlation test (Spearman's r_s) was used for determining significant correlation coefficients of elements among the different tissues and inter-element correlations within the same tissue. The significance levels of correlations were Sidak-adjusted for pair-wise comparisons. Element ratios were created by dividing element concentrations in the meat by the same element concentrations in the rest of the studied tissues and mean values of the element ratios were obtained. Mean, median, p-value, correlation coefficients and element ratios were not calculated in cases in which the detection percentage was below 50%. The level of statistical significance was set at $p < 0.05$ in all performed analyses.

Calculation of estimated human weekly/monthly toxic elements intake from reindeer

A scenario based on data from a questionnaire on the Population-based Health and Living Conditions in areas with Sami and Norwegian populations – The SAMI-NOR Study – was used to estimate reindeer meat and liver intake (19). This estimation was used to determine toxic elements exposure on the basis of weekly human reindeer meat and liver intake within this population. This was then compared with the PTWI/PTMI limits in order to assess the different toxic elements exposure for a standard 60 kg human body weight, as set by FAO/WHO–JECFA (17). The percentiles 25, 50 and 75% were used to determine the distribution of meat intake, in which 25% was given to low (23 g/week), 50% to medium (38 g/week) and 75% to high consumption (70 g/week). Due to very low liver consumption, the mean consumption frequency of once in a month or more was used to

determine high liver intake (61 g/week). The proportion of the population with high liver intake was about 2% (total $n = 12,899$). The estimated toxic elements concentrations on the basis of human weekly/monthly reindeer meat and liver intake (EHTI) were calculated according to the following formula:

$$\text{EHTI } (\mu\text{g/kg human body weight}) = I \times T/S.$$

where:

(I) is the amount of estimated weekly edible tissue intake in grams, (T) is toxic element concentration in the meat tissue per gram and (S) is the standard human body weight equal to 60 kg. In addition to the PTWI/PTMI, the ML of toxic elements in a reindeer body was directly calculated per kilogram of meat/liver. Since there are no official ML for toxic elements in the meat and liver of cervine species, the available ML for bovine animals' meat and liver were used (16).

Results

The percentage of samples within the detection limits (% detected), mean, median ng/g wet weight (ww) and range are presented in Table I. The ratios of element concentrations between liver and the rest of the studied tissues are presented in Table II. The toxic element correlations between liver and the rest of the studied tissues are presented in Table III.

Cadmium (Cd)

Cd was detected in 100% of tissues studied, with the highest concentration in liver (mean 653.7, median 563.9 ng/g ww) and the lowest in meat (mean 1.9, median 1.2 ng/g ww, $p < 0.01$). Cd was significantly higher in tallow than in bone marrow ($p < 0.05$). In addition, Cd concentrations in meat and liver were significantly positive correlated (Spearman's $r_s = 0.72$, $p < 0.01$).

Lead (Pb)

Pb was detected in 100% of liver, tallow and bone marrow samples and 83% of meat samples. The highest Pb concentration was measured in liver (mean 272, median 247.8 ng/g ww) and the lowest in meat (mean 7.9, median 7.6 ng/g ww), $p < 0.01$. No significant correlation was found between Pb concentrations in meat, liver or any of the other tissues.

Arsenic (As)

As was detected in all tissue samples with the exception of bone marrow in which only 44% of the concentrations were above the LOD. Liver had the highest As concentration (mean 24.1, median 13.1 ng/g ww). A significant positive correlation coefficient (Spearman's $r_s = 0.71$, $p < 0.01$) was found for As in meat and liver. The As was not significantly inter-correlated with any other element in the 4 tissues studied.

Table I. Levels of toxic elements (ng/g ww) in meat, liver, tallow and bone marrow of semi-domesticated reindeer

Element	Meat (n = 29)					Liver (n = 29)					p ^b	LOD
	% Detected ^a	Mean	Median	Min	Max	% Detected	Mean	Median	Min	Max		
Cd	100	1.9	1.2	0.6	7.1	100	653.7	563.9	174.7	2,186.2	*	0.37
Pb	83	7.9	7.6	1.8	18.1	100	272.0	247.8	144.5	522.7	**	3.61
As	100	19.7	11.6	1.3	82.6	96	24.1	13.1	0.6	156.6	*	1.13
Ni	48	— ^c	— ^c	19.7	101.7	72	50.5	42.5	19.7	185.9	— ^c	39.46
V	48	— ^c	— ^c	0.1	4.9	100	13.1	9.6	4.6	43.7	— ^c	0.24
Tallow (n = 15)						Bone marrow (n = 9)						
Cd	100	5.7	5.7	2.5	11.9	100	3.8	3.8	2.5	5.5	*	0.37
Pb	100	28.6	26.6	11.3	64.4	100	21.8	22.6	7.0	36.3	n.s	3.61
As	87	2.1	1.9	0.6	4.0	44	— ^c	— ^c	<1.1	78.1	— ^c	1.13
Ni	13	— ^c	— ^c	19.7	326.1	100	285.6	253.5	46.5	653.0	— ^c	39.46
V	93	1.3	0.9	0.1	6.0	100	2.6	1.0	0.4	7.8	n.s	0.24

^aPercentage of samples within the detection limits.

^bLevel of significance: * <0.05, ** <(0.01), n.s (not significant).

^cNot calculated because more than 50% of the values were below the limit of detection (LOD).

Note: To detect significant differences, meat was tested against liver, and tallow was tested against bone marrow.

Nickel (Ni)

Forty-eight percent of Ni concentrations in meat and 13% in tallow were above the LOD. All Ni concentrations in bone marrow were above the LOD, whereas 72% of concentrations in liver were above the LOD. The concentration in bone marrow was 6 times higher than that in liver, with mean 285.6, median 253.5 and mean 50.5, median 42.5 ng/g ww, respectively. Due to a low detection percentage, the nickel mean ratio was only calculated between liver and bone marrow (0.38).

Vanadium (V)

Forty-eight percent of V concentrations were above the LOD in meat, none were under the LOD in bone marrow, while 93% were above the LOD in tallow. V was the least abundant element in the different tissue samples, with a range between 0.93 and 9.57 ng/g ww. The V concentration was highest in liver (mean 13.11, median 9.57 ng/g ww) and lowest in tallow (mean 1.32, median 0.93 ng/g ww).

Scenarios for estimated human weekly and monthly toxic elements intake from reindeer

Scenarios for estimated human weekly and monthly toxic elements intake from reindeer meat and liver were calculated according to the formula given previously in Materials and methods section on the basis of the following meat and liver consumptions (weekly for As and Pb, and monthly for Cd): (a) Weekly/monthly meat consumption: low (23/92 g), medium (38/152 g) and high (70/280 g), respectively. (b) Weekly/monthly liver consumption: 61 and 244 g, respectively. The estimated human toxic element intake values are presented in Table IV, together with the PTWI and PTMI limits (see calculation of estimated human toxic elements intake from reindeer under Materials and methods section).

Discussion

Levels of the studied selected toxic elements in reindeer were low compared to maximum levels for bovine animals set by the EC, except for Cd in liver. Cadmium

Table II. Ratio of toxic element concentrations between liver and the rest of the studied tissues

	Mean toxic element ratio ^a				
	Cd	Pb	As	Ni	V
Liver/meat	410.22	51.48	1.37	— ^b	— ^b
Liver/tallow	91.07	12.13	7.97	— ^b	31.21
Liver/bone marrow	131.82	18.46	— ^b	0.38	12.34

^aToxic element ratio was calculated by dividing the individual concentration of the specific element in liver by that of the same element in the other tissue for the same individual. Then, the mean ratio of all these ratios for each toxic element was obtained and reported, aiming to give an idea about toxic element concentrations in liver when directly compared to the other tissue.

^bThe toxic element ratio between liver and the rest of the studied tissues was not calculated in cases in which at least one of the ratio components had a percentage of samples within the detection limits that was below 50%.

Table III. Toxic element correlations between liver and different tissues from reindeer

Element	Correlation coefficient					
	Meat–liver	p-value	Tallow–liver	p-value	Bone marrow–liver	p-value
Cadmium	0.72	<0.01	0.38	n.s ^a	0.61	n.s ^a
Lead	0.09	n.s ^a	0.18	n.s ^a	–0.26	n.s ^a
Arsenic	0.71	<0.01	0.61	n.s ^a	–0.79	n.s ^a
Nickel	– ^b	– ^b	– ^b	– ^b	0.07	n.s ^a
Vanadium	– ^b	– ^b	–0.15	n.s ^a	–0.02	n.s ^a

^aNot significant.^bThe correlation was not calculated in cases in which at least one of the correlation components had less than 50% of samples within the detection limits.

exceeded the EC's ML for bovine animals in 52% of the liver samples. This level would not represent a potential health risk to the population as the estimated monthly Cd intake from liver was well below the PTMI limit set by JECFA. The low Cd intake from liver was certainly because of the very low reindeer liver consumption. This would further indicate a necessity to not use the ML alone when relating toxic element levels in reindeer and games to human intake of such elements. The tolerable intakes set by FAO/WHO–JECFA would be more appropriate to use when dietary frequency could be estimated through questionnaire data.

Cadmium

The Cd concentration in reindeer liver from this study was twice the amount detected by Bernhoft et al. (20), nearly half the amount reported by Frank (21) and comparable with those detected elsewhere (22,23), in which levels were 2–3 times of those reported in moose and sheep from same area. Our median Cd concentration of 1.2 ng/g ww (equivalent to 0.0012 mg/kg ww) in reindeer meat was much lower than the EC's ML for Cd in the meat of bovine animals, sheep, pig and poultry of 0.05 mg/kg ww. However, the median Cd level of 563.9 ng/g ww in reindeer livers was 60 ng/g higher than the

ML of 0.50 mg/kg ww set for liver (16). Moreover, the Cd level exceeded the EC's ML for bovine animals in 52% of the liver samples (n = 29) collected. High Cd concentrations from reindeer and caribou livers have also been shown previously (13,23–27).

The FAO/WHO–JECFA has recently set the PTMI limit at 25 µg/kg body weight (standard human = 60 kg) (14). The estimated monthly human Cd intake from reindeer meat for low, medium and high consumption, as well as from reindeer liver (high consumers), was well below the PTMI limit for Cd. The high reindeer meat and liver consumption is relevant to reindeer herders and hunters. In order to reach the PTMI for Cd when liver is the issue of concern for high consumers, an amount of 2.7 kg per month needs to be eaten. Since the PTMIs for toxic elements have been established to take into account the total intake of such elements from different sources/type of diets, it is important to take into account Cd intake from smoking and other type of diets.

Lead

The level of liver Pb from this study is comparable to those reported by Sivertsen et al. (22), which are within the range reported elsewhere (20,24). The Pb level in meat was much lower than in liver and was comparable with

Table IV. Estimated human toxic elements intake from reindeer meat and liver

Element	Human toxic elements intake (µg/kg body weight)				
	Meat			Liver	PTWI/PTMI ^a
	Low (23 g/week)	Medium (38 g/week)	High (70 g/week)	High (61 g/week)	
Cd ^b	0.002	0.003	0.006	2.293	–/25
Pb ^c	0.003	0.005	0.009	0.252	25/–
As ^c	0.005	0.007	0.014	0.013	15/–

^aPermissible tolerable weekly intake/permissible tolerable monthly intake (µg/kg body weight).^bMonthly toxic element intake.^cWeekly toxic element intake.

Note: For the meat intake, low is (the 25 percentile), medium (the 50 percentile) and high (the 75 percentile), while the high liver intake represents the mean consumption frequency of once a month or more based on data from the SAMINOR-Study (19).

the level reported by Rintala et al. (24). The reported liver concentration of lead from north Norwegian sheep was 28% lower than the value detected from reindeer livers in the present study (28). The concentration of lead levels in livers detected in this study was comparable to those reported by Eriksson et al. (29). However, Eriksson et al. reported nearly double the maximum level (0.99 mg/kg ww) in their range than the one we detected of 522.7 ng/g ww. The median liver Pb of 247.8 ng/g ww in the present study was much lower than that of 10 µg/g ww, which was reported to be consistent with the poisoning level in most animal species (30). Lead was found to be significantly correlated with As, Co and Cu in liver and with As and Cu, in meat from Spanish cattle (31,32). Even so, we could not see such correlations in this study.

The respective meat and liver Pb concentrations of 7.6 and 247.8 ng/g ww in this study were much below the EC's ML for the meat and offal of bovine animals, sheep, pig and poultry, which were set at 0.10 and 0.50 mg/kg ww, respectively (16). The PTWI limit for Pb of 25 µg/kg body weight was recently withdrawn by FAO/WHO–JECFA. No new permissible tolerable intake limit for Pb was established. Nevertheless, weekly human Pb intake from reindeer liver, which was the highest intake compared with meat, was 100 times lower than the previous PTWI limit (Table IV).

Arsenic

Liver values reported elsewhere (20,22,33) were within our range of 0.6–156.6 ng/g ww. The extremely large arsenic range measured in this study could be due geography and need to be investigated further with more animals and involvement of more herding districts. Sivertsen et al. also reported significant inter-element correlations in reindeer between As and Co and Cu and Ni (22). Arsenic was significantly positive correlated ($r_s = 0.71$, $p < 0.01$) between meat and liver in this study. Such a correlation could be beneficial in estimating As levels in an animal body, using liver as an indicator/biomarker of As body burden. Estimated weekly As intakes from reindeer meat and reindeer liver were well below the PTWI limit for As of 15 µg/kg body weight (standard human = 60 kg) set by FAO/WHO–JECFA (15). As, which is antagonistic to the essential elements of iodine (I) and selenium (Se), accumulates in liver, kidney, skin and hair (34,35). Both organic and inorganic forms are toxic to animal.

Nickel

The median Ni liver concentration in this study was comparable with that formerly reported by Bernhoft et al. (20) and within the range formerly reported by Sivertsen et al. (22,33). The level of Ni in Finnish reindeer livers, which was lower than the level detected in our study, was found within the same range (0.01–0.03 mg/kg ww) of those from Finnish cattle and pigs (24).

In previous studies, nickel concentrations in reindeer from Sør-Varanger in northern Norway were associated with atmospheric pollution from Ni smelters in 2 Russian towns (Nikel and Zapoljarnij) close to the Norwegian border in which concentrations in reindeer were higher than those in moose and sheep from the same area (22).

Vanadium

Meat, liver and bone marrow concentrations of V in this study were comparable to those reported from cattle and pigs (36). No clear regulation of V has been established in human; however, a concentration of 100 µg/day has been reported as an estimated safe daily intake (37).

Element correlation between liver and meat

Still, few studies have looked at the correlation of elements between meat–liver and meat–kidneys, despite the fact that liver and kidneys are used in food safety aspects as indicators of toxic elements in meat. Only Cd and As were statistically significantly correlated between meat and liver in the present study (Table III), whereas a significant correlation for Pb between liver and meat has been reported elsewhere (38).

Conclusions

Liver had the highest toxic element concentrations with the exception of Ni, which was highest in bone marrow. The correlations among the detected elements between liver and meat were observed for Cd and As. Therefore, liver is not a good indicator for lead in meat.

Based on the measured levels of the present studied elements and their relation to the EC's ML and the PTWI/PTMI limits, we could infer that the consumption of reindeer meat is not associated with any health risk for consumers. The Cd level exceeded the EC's ML for bovine animals in 52% of the liver samples. Nonetheless, the monthly Cd intake of 2.3 µg/kg body weight from liver was well below the PTMI of 25 µg/kg body weight set by JECFA. In the present study, Cd, Pb, As and Ni levels from reindeer seem not to differ much from previous Norwegian studies.

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